# ABSTRACT

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Title of diploma thesis: Cloning, expression and purification of human AKR1C4.

AKR1C4 is one of four enzymes in men belonging to subfamily of aldo-keto reductases AKR1C. It is monomeric cytosolic protein with length of 323 amino acids expressed in liver. It plays an important role both in metabolism of endogenous and exogenous substances. It is involved in the metabolism of steroid hormones and bile acids and many drugs, for example tibolone and naltrexone. It also plays a role in activation of some cancerogenic substances, e.g. PAHs.

cDNA of enzyme was delivered in cells of E. coli DH10B, in pDNR-LIB vector. After lysis of cells and isolation of plasmid, the coding sequence was amplified by PCR. Afterwards it was ligated into vector pET-28b, thanks to added restriction sites for Nde I and Xho I endonucleases in designed PCR primers. The recombinant plasmid prepared by this way was transformed by heat shock to cells E. coli HB101. After amplification of ligated plasmid it was transformed to E. coli BL21. Adjusted cells BL21 were used for expression of the protein. IPTG was used as induction reagent for overexpression. Pure expressed recombinant protein AKR1C4 was purified by ÄKTA purifier using chelating columns and its purity was verified by SDS-PAGE electrophoresis. Enzyme together with cell cultures of HB101 and BL21 with ligated plasmids have been stored for next use. One part of stored enzyme was cleaved off His-tag. For both sorts of enzyme, with and without His-tag, their activity against oracin was measured to verify their proper function.